Trace Chemical Detection Through Vegetation Sentinels and Fluorescence Spectroscopy

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ABSTRACT

Detection of environmental contaminants through vegetation sentinels has long been a goal of remote sensing scientists. A promising technique that should be scalable to wide-area applications is the combined use of genetically modified vascular plants and fluorescence imaging. The ultimate goal of our research is to produce a bioreporter that will express fluorescence when encountering nitro-aromatic compounds such as munitions contaminants. To test the recovery of *gfp,* our study used tobacco plants (*Nicotiana tabacum*) that were genetically modified to express the *m-gfp5-ER* variant of the green fluorescent protein (*gfp*) in conductive tissues. Induction of the *gfp* was stimulated by the uptake and translocation of a systemic organo-phosphate pesticide. The first objective of the study was to detect the induced gfp emission in plants exposed to the pesticide. The second objective of this effort was to use a field spectrometer and imaging system to determine if the fluorescence signature (from the induced plants) was spectrally separable from negative controls and permanently expressing plants. Concurrent research is underway to optimize the induction specificity of the *gfp* for a variety of target materials (e.g., TNT, RDX, HMX). Here, we report results from a Phase I small-business technical transfer research grant (STTR) conducted in Edgefield, SC. Our tests showed that *gfp* could be detected by spectrofluormetry and laser imaging and that expressing plants produced approximately three times the fluorescence at 510 nm as the negative control. Correlation and agreement between the non-imaging and imaging spectrometer also showed the optimal excitation wavelength (*gfp* absorption maxima) to be between 390 nm and 410 nm. When matched with the emission wavelength, these numbers represent a broad Stoke's shift of almost 100 nm that is optimal for *gfp* signal recovery.

INTRODUCTION

The detection of unexploded ordnance (UXO) and landmines is a major concern for ground forces, land reclamation and commercialized infrastructure projects. In addition, mandated clean up and reclamation of military bases requires a robust method to detect contaminants related to UXO and explosive waste materials. The Base Realignment and Closure Act (BRAC) and the efforts for environmental recovery of Formerly Used Defense Sites (FUDS), has demonstrated that many of these military bases undergoing recovery possess range sites littered with the hazards of unexploded ordnance (UXO), explosives-related waste, and other soil-based contaminants. Other issues include the vast number of landmines in regions around the world that threaten human health and the

socio-economic structure of many nations. Additionally, buried ordinance and explosives limit access to vast natural resources (CalEPA, 2000).

Safe, expedient, and cost effective remediation necessitates technological developments that can detect UXO hazards over vast areas, sometimes involving hundreds of square miles. Detection by an airborne platform is preferred alternative over the current state-of-the-art that involves discovering ordinance using specialized technicians on foot. Technicians must use manual means to tediously explore extensively contaminated areas. In many instances, this work is not only cost-prohibitive but orders of magnitude more dangerous than a remote detection technology.

The use of vegetation as sentinels to indicate presence/absence of contaminants provides an ideal mechanism for a wide-area detection scenario. With the exception of extreme arid, polar, and oceanic environments, vegetation is the dominant cover-type viewed from remote sensing platforms. Plants also have the ability to interrogate their environment spatially and temporally through roots and leaf stomata. It has been demonstrated that visible, near-infrared, and short-wave infrared spectral cues produced by natural vegetation can indicate the presence or absence of (toxic) materials (Milton et al., 1991; Vane and Goetz, 1993). However, research has shown that it is difficult to positively attribute spectral phenomena to a specific plant-contaminant interaction. In most cases, morphological and photosystem stress cues are difficult to correlate with specific toxicants, particularly when both organic and inorganic compounds are present (Carter et al., 2000). Furthermore, quantification of toxicants (in mass equivalent levels) that are available for uptake is nearly impossible remotely.

One solution to this problem is to modify a desired plant species through mutagenesis or genetic engineering to produce a diagnostic spectral response only when that plant comes into contact with a particular target material. A technique that has received the greatest amount of interest is the insertion of genes that promote the expression of the green fluorescent protein (*gfp*). Using genetic engineering, *gfp* can be inserted into specific tissues of plants, optimizing signature recovery. The protein originates from the jellyfish *Aequorea victoria*, and is often used as a marker for the non-destructive visualization of molecular biological processes involving both eukaryotic and prokaryotic cells. For the indication of contaminants, fluorescent protein expression is induced by specific (gene) promoters; linked to the synthesis or hyper-accumulation of a target material or substrate. Depending upon the variant, *gfp* produces a diagnostic fluorescence emission at ~ 509 nm that can be detected in dark field or separable from background light (Niwa et al. 1999; Chiu et al. 1996). Current research is examining application of proteins that emit in the yellow and red spectral regions (i.e., yellow fluorescent protein (*yfp*) and red fluorescent protein (*rfp*)). Many of the biochemical pathways for assimilation and bio-degradation that can foster fluorescence have been extensively studied by Meagher et al. (2000). As sentinels, plants offer the best buffer to maintain stability of fluorescent proteins that can be sensitive to fluctuations in charge state (Smith, 2002).

METHODS

Testing for the Phase I small-business technical transfer research grant (STTR) was conducted at NEWTEC Inc. of Edgefield, SC during May 20-22, 2002. NEWTEC provides a 1,500 acre federal and state licensed explosive test facility with approved storage magazines and an inventory of explosive materials in support of the original LIFI/bioreporter development program. NEWTEC coordinated and developed all previous permitting reports and gained approvals for deployment of the technology at its facility via the US Environmental Protection Agency, and South Carolina Department of Health and Environmental Control.

The genetically modified plants were tobacco (*Nicotiana tabacum*), engineered with the modified *gfp* gene (*m-gfp5-ER* or MGFP*)* through agrobacterium mediated transformation. Wild type tobacco plants were utilized as negative controls (NC population); plants engineered for permanent *gfp* expression (constitutive expressers, MGFP population) served as positive controls. Induction of *gfp* in test plants was mediated by the translocation and metabolism of a systemic organo-phosphate pesticide. Expression of fluorescence occurred in both leaves and conductive tissues.

The spectroscopic properties of the tobacco plants were investigated with a variety of instruments and methods. Reflectance was measured with the Analytical Spectral Devices (ASD) Field Spec Pro. Fluorescence detection hardware consisted of a portable *gfp* Meter, a JY Horiba Fluoromax-3 spectrofluorometer, and Laser Induced Fluorescent Imaging and Spectral (LIFI/LIFS) sensing equipment.

RESULTS

Visible – near infrared reflectance signatures were collected using the ASD FieldSpec radiometer on May 20 and May 22. The spectral response of the individual signatures varied as a function of canopy dimensions (i.e., amount of potting visible to the radiometer) and canopy geometry. Comparisons between negative control plants and constitutive expressers showed no substantial difference in the specific population average reflectance spectrum (Figure 1). The only notable difference is a few percent offset between 750 and 900 nm, which is negligible when considering the $\pm 1\,\sigma$ error bars. The spectral "spikes" at 925, 1900 and 2500 nm are due to atmospheric absorption that was not removed or smoothed by the post processing software.
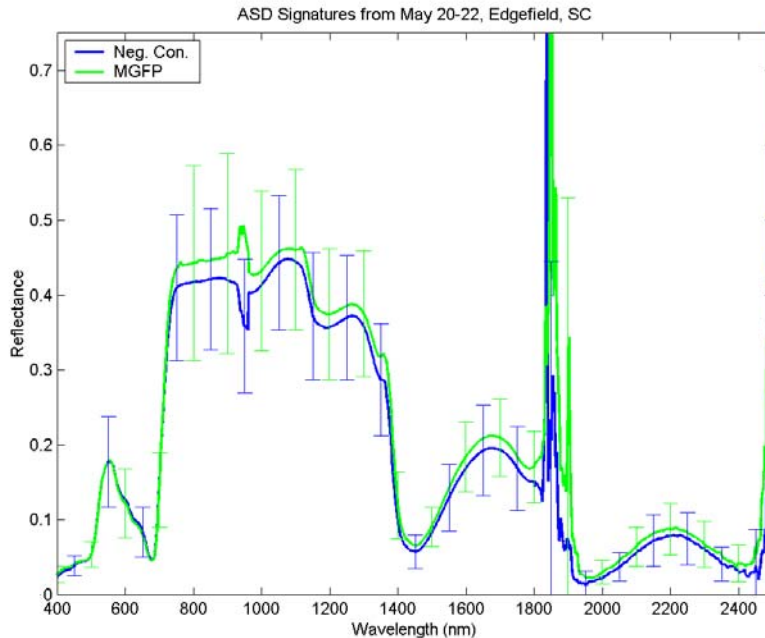
Figure 1. Reflectance signatures from NEWTEC, South Carolina. Blue is negative control, green is MGFP population. Error bars represent ± one standard deviation. Nineteen samples were used for the negative control, 17 for the MGFP.

Fluorescence spectra obtained using the Fluoromax-3 was successful in detecting enhanced *gfp* expression from the MGFP population. Figure 2 shows the excitation spectra collected at 510 emission. The 510 nm data represent excitation potential at the *gfp* emission maximum. Figure 2 shows that the negative control and MGFP plants separate at excitation wavelengths between 390 and 420 nm. The maximum separation between the two curves is found at approximately 410 nm, with the MGFP plants fluorescing almost three times as many photons as the negative control plants. Separation is good in this spectral region as shown by the one standard deviation error bars. This data suggests that the optimum excitation to differentiate the negative control plants from the constitutive expressers and, potentially, the experimental plants, is an excitation source at 400 nm.

Figure 3 shows the emission scan collected at 400 nm excitation for the control and MGFP plants. As indicated by Figure 3, maximum spectral separation is evident in the 510 nm emission range. At wavelengths longer than 550 nm, the signatures converge and separation is no longer possible. This figure shows that the emission at 510 nm from the MGFP plants is roughly three times that of the negative control plants as shown by the emission scan in Figure 2. One standard deviation error bars show the spectral difference is significant.

Figure 4 shows the LIFS-derived data demonstrate that *gfp* modified plants are spectrally separable from the control plants, most strongly at the *gfp* emission maximum of 510 nm (with an excitation of 400 nm). The LIFS data were collected at a distance of

approximately one meter, demonstrated the concept of stand-off detection. These data corroborate the data acquired with the *gfp* meter as well as the Fluoromax-3. Leaf canopies were interrogated at 400 and 355 nm excitation. A small difference in emission intensity is observed at 355 nm excitation between MGFP and NC plants (not shown) but the difference in fluorescence emission is much more distinct when the excitation is at 400 nm.
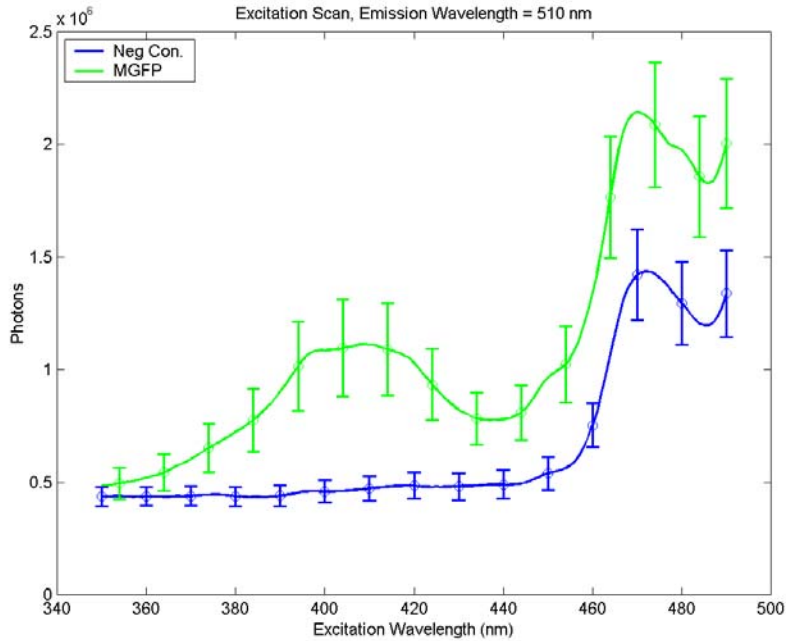

Figure 2. Fluorescence excitation signatures for negative control and MGFP plants. Emission wavelength = 510 nm. Eight samples were used to create each of the signatures.
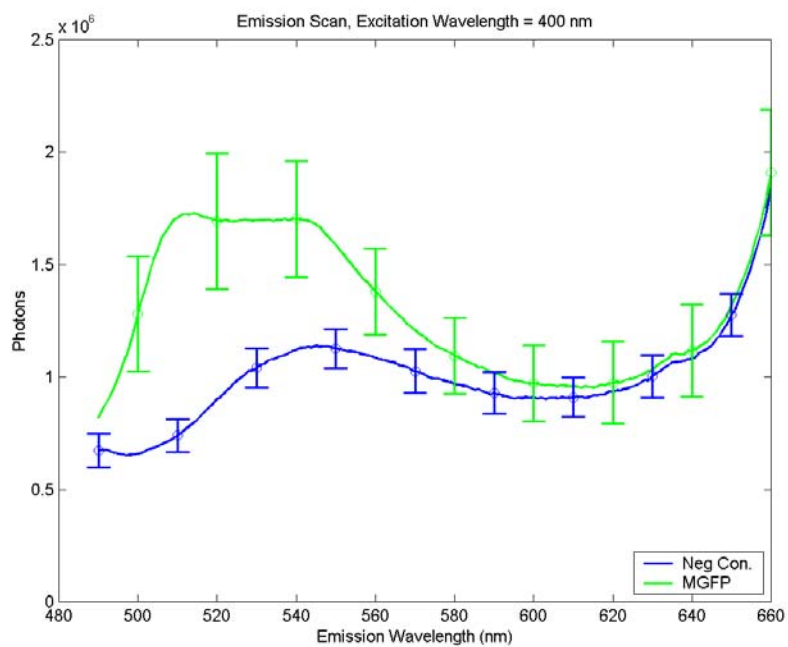
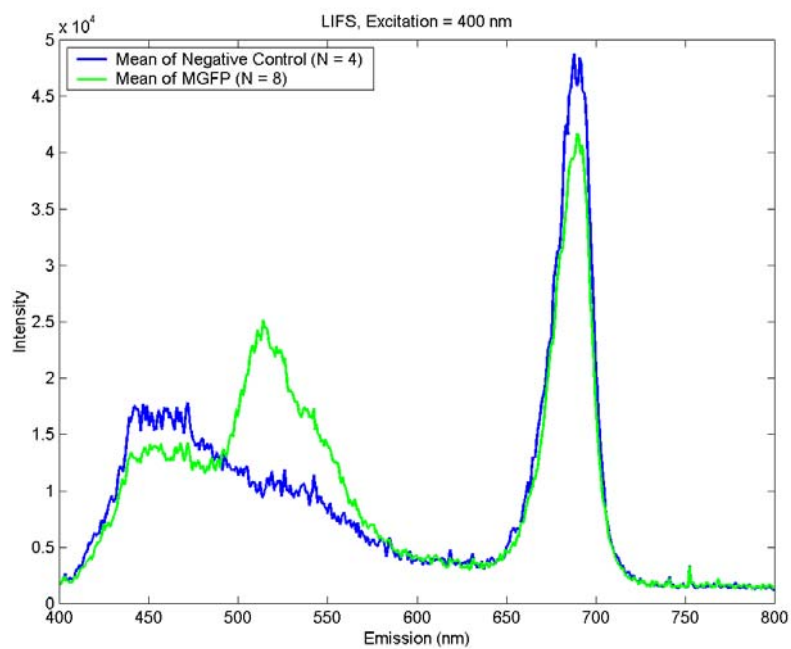**Figure 3. Emission spectra from May 22. Excitation wavelength = 400 nm.**

Figure 4. LIFS signatures from control and *gfp* expressing plants.

Figure 5 shows the results obtained from the Laser Induced Fluorescence Imaging system. These data were also collected at a one meter stand-off distance. The laser light was pulsed at an excitation wavelength of 355 nm. An optical receiver with a bandpass filter of 510 nm captured the resulting fluorescence. An algorithm was used to remove the green background chlorophyll fluorescence. The resultant images show that MGFP plants produced a detectable response. This result is encouraging when considering that these plants were excited at a non-optimal wavelength of 355 nm.
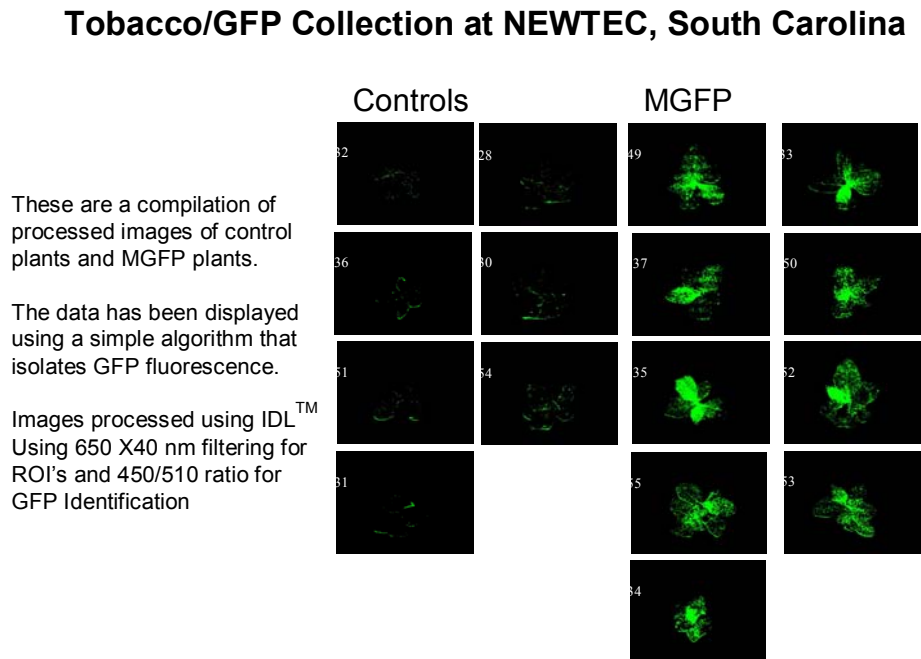
Tobacco/GFP Collection at NEWTEC, South Carolina

Figure 5. LIFI images of negative control (left) and expressing tobacco plants (right).

CONCLUSIONS

The focus of the research effort was to generate and evaluate fluorescent signatures of genetically modified plants and to tailor the plant fluorescence to optimize the use of existing passive or active imaging technology from an airborne platform. The purpose of this test was to perform a field demonstration and proof of concept that plants genetically programmed to express were detectable and separable from negative control plants.

Fluorescence spectra obtained from the Fluoromax-3 was successful in detecting the *gfp* expression from the constitutive expresser population at the *gfp* wavelength of 510 nm. The maximum separation between the two curves is found at an excitation wavelength of approximately 410 nm, with the MGFP plants fluorescing almost three times as many photons as the negative control plants. Separation is good in this spectral region as shown by the error bars.

The results found by the Fluoromax spectrofluorometer were supported by the stand-off LIFS and LIFI systems. The LIFS also detected a factor of three intensity difference between the negative control and expressing plants. While numerical measurements from the LIFI system were not available, the MGFP plants were separable from the NC plants after the removal of green chlorophyll fluorescence.

The test showed genetically modified plants expressing *gfp* can be successfully interrogated and detected with spectroscopy and stand-off fluorescence systems. Research is actively underway to develop a host of different plants that possess higher selectivity and express (*gfp)* only after synthesis of specific contaminants (such as TNT). If plants can be developed to respond (by fluorescence) to a particular contaminant at intensities seen in the MGFP population, then detection of target contaminants should be feasible by fluorescence remote sensing.

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